P-ISSN : 0215 - 4706
E-ISSN : 2460 - 6944

FLORIBUNDA
Jurnal Sistematika Tumbuhan

AUTHENTICATION OF THREE WAX APPLES CULTIVARS (*Syzygium samarangense* (Blume) Merr. & L.M. Perry) BASED ON MORPHOLOGICAL CHARACTER AND FRUIT METABOLITE PROFILE

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ABSTRAK

Annisa Nur Rachmah, Asri Febriana, Niken Kusumarini, Eka Oktaviani, Arnia Sari Mukaromah 2023. Autentikasi Tiga Kultivar Jambu Semarang (*Syzygium samarangese* (Blume) Merr & L.M. Perry Berdasarkan Karakter Morfologi dan Profil Metabolit Buah. *Floribunda* 7(2): 64–74 — Jambu semarang merupakan salah satu buah unggulan Demak, khususnya jambu semarang kultivar 'Citra', 'Delima' dan 'Madu Deli Hijau'. Penelitian tentang ketiga kultivar buah tersebut masih terbatas. Oleh karena itu, penelitian mengenai autentikasi kultivar jambu semarang berdasarkan karakter morfologi dan profil metabolit yang terdapat dalam satu lokasi penting untuk dilakukan. Tujuan penelitian ini yaitu mengidentifikasi karakter morfologi, profil metabolit buah, dan menganalisis kekerabatan fenetiknya. Pengambilan sampel di Desa Boyolali, Kabupaten Demak. Tahapan penelitian ini meliputi karakterisasi morfologi pada organ batang, daun, buah dan biji, analisis kekerabatan dengan MVSP, analisis profil metabolit buah menggunakan GC-MS serta penentuan karakter morfologi dan profil metabolit penanda dengan PCA. Hasil penelitian menunjukkan adanya keanekaragaman morfologi dan variasi profil metabolit pada ketiga kultivar. Analisis kekerabatan berdasarkan karakter morfologi dan profil metabolit menghasilkan pola pengelompokan yang berbeda. Jambu semarang 'Madu Deli Hijau' dan 'Citra' berkerabat dekat berdasarkan karakter morfologi dan gabungan karakter morfologi dan profil metabolit. Karakter morfologi dalam autentikasi jambu semarang 'Citra' (warna eksokarpium merah gelap), jambu semarang 'Delima' (warna eksokarpium merah cerah, permukaan buah licin dan rata, bangun buah melonceng), pada jambu semarang 'Madu Deli Hijau' (bangun daun memanjang dan eksokarpium bewarna hijau dengan semburan merah muda). Autentikasi kemungkinan profil metabolit penanda pada jambu semarang 'Citra', 'Delima' dan 'Madu Deli Hijau' berturut-turut sebanyak 10, 7 dan 5 senyawa.

Kata kunci: Jambu semarang, kekerabatan, kultivar, morfologi, profil metabolit

Annisa Nur Rachmah, Asri Febriana, Niken Kusumarini, Eka Oktaviani, Arnia Sari Mukaromah 2023. Authentication of Three Wax Apples Cultivars (S*yzygium samarangense* (Blume) Merr. & L.M. Perry) Based on Morphological Character and Fruit Metabolite Profile. *Floribunda* 7(2): 64–74 — Wax apple is one of the superior fruits of Demak, especially cultivars 'Citra', 'Delima' and 'Madu Deli Hijau'. Research on the wax apple of the three cultivars is still limited. Therefore, research on the authentication of wax apple cultivars based on morphological characters and metabolite profiles present in one location is important to do. The aims of this study were to identify morphological characters and analyze metabolite profiles, to analyze relationship and to identify morphological characters and metabolite marker profiles in the authentication activities of three wax apples cultivars. Sampling was in Boyolali Village, Demak Regency. The stages of this research included morphological characterization of stem, leaf, fruit and seed organs, phylogeny analysis with MVSP, metabolite profile analysis using GC-MS, and determination of morphological characters and metabolite profiles with PCA. The results showed that there were morphological diversity and metabolite profile variations of the three cultivars. Relationship analysis based on morphological characters and metabolite profiles resulted in different grouping patterns. 'Madu Deli Hijau' and 'Citra' are closely related

based on morphological characters and a combination of morphological characters and metabolite profiles. Morphological characters in the authentication of the 'Citra' (dark red exocarp color), 'Delima' (bright red exocarp color, smooth and flat fruit surface, jagged fruit shape), in 'Madu Deli Hijau' (elongated leaf shape and exocarp is green with a pink tinge). Authentication of probable marker metabolite profiles on 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples of 10, 7 and 5 compounds.

Keywords: Wax apple, relationship, cultivar, morphology, metabolite profile

Wax apple is a tropical non-climacteric fruit that grows in Asia, including Indonesia, and is a member of family Myrtaceae and support human nutritional values and health (Mukaromah, 2020; Pujiastuti, 2015). Based on taxonomists, wax apple is divided into two subgroups, namely *Syzygium aqueum* (Burm. f.) Alston and *S. samarangense* (Blume) Merr. & L.M. Perry. *Syzygium aqueum* has relatively small fruit sizes and a sour taste, while *S. samarangense* has larger fruit sizes and sweet tastes (Pujiastuti, 2015). Therefore, *S. samaranganse*, commonly called wax apple, is more widely cultivated by farmers. Based on the shape, color, taste, and size of the fruit, the wax apple has various cultivars (Widodo *et al.,* 2018). Demak is known as a wax apple-producing area, especially for the cultivars 'Citra', 'Delima' and 'Madu Deli Hijau' which are Demak's superior fruits.

Cultivar variation in the wax apple is a form of genetic diversity that can affect the morphological characters displayed. Therefore, it is necessary to carry out morphological characterization activities to make it easier to differentiate cultivars, determine authenticity, and detect diversity based on phenotypes. In addition to influencing morphological characters, cultivar variations can affect the metabolite content of a plant. This is because genetic factors can affect the biosynthetic process of a plant (Maruzy *et al*., 2020). Metabolites are bioactive substances that have multiple structures, so that authentication with metabolite profiles can be used as markers to differentiating samples (Doh *et al*., 2020). Fitmawati *et al*., (2021) research stated that *Mangifera sumatrana* and *Mangifera laurina* are not the same. These findings reveal the significance of metabolite content as a taxonomic marker. Therefore, authentication using morphological characters need to be combined with other approaches, such as metabolite profile analysis, to determine the presence of marker compounds in a plant.

Research on the cultivars 'Citra', 'Delima', and 'Madu Deli Hijau' is still limited. Cultivar diversity in the wax apple is one of the genetic resources that need to be analyzed. Therefore, it is important to conduct this research to obtain information on the morphological characters and metabolite profiles of the three cultivars of wax apple, their phenetic relationships, and the morphological characters and metabolite profiles that can be used in the authentication activities of the three wax apple cultivars.

MATERIALS AND METHODS

Materials

The materials used in this study were the stem, leaves, fruit, and seeds of three cultivars of wax apples from Boyolali Village, Demak Regency, alcohol, millimeter paper, methanol for ethanol, chloroform, silica gel, distilled water, aluminum foil, and paper.

Morphological character analysis

Three accessions were taken for each cultivar, and then the morphological characters of stems, leaves, fruit, and seeds were measured and recorded. For taste and texture parameters of fruits used organoleptic analysis was carried out on seven panelists with a predetermined score. Data is processed in binary form using Microsoft Excel 2010. Relationship analysis according to UPGMA (*Unweighted Pair Group With Arithmetic Mean*) with the SSM (*Simple Matching Coefficient*) method using MVSP 3.2 The results obtained are in the form of a phenogram, and a similarity index were described. The determination of marker morphological characters was carried out descriptively by comparing characters that are constant and can be used as a differentiator between three cultivars.

Metabolite Profile Analysis

Samples of 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples were perfectly ripe. The fruit is washed, the seeds are removed and then cut into thin pieces and dried for three days at 60 ºC until the fruit is dry. The dried fruit is then mashed. Maceration was carried out on 1 g of sample using 10 ml of methanol: chloroform: distilled water (5:2:2) for 24 hours. The filtrate obtained was transferred and remediated using the same solvent. Samples in the form of extracts were evaporated using a rotary evaporator and freeze dried. 1 mg sample was analyzed using GC MS using a solvent combination of 1 mL methanol: chloroform (7:2). The fruit extracts were processed into GC-MS after the machine was stabilized with an initial column temperature of 500 °C for 2 minutes. After that, the

column temperature was increased from 5 ºC/ minute to 200 ºC and then held for 10 minutes. The column temperature was increased from 10ºC/ minute to 250ºC/minute. The injector is set to split ratio mode (21:1). The Thermo Scientific TG-5MS column (semi polar column) is used with dimensions of 30m length, 0.25mm I.D, and 0.25µm film. The carrier gas used is helium with a flow rate of 1 mL min-1. As much as 1 µL was injected and run for 30 minutes. The chromatograms and MS data obtained were then downloaded and analyzed.

Data obtained was compared manually with the NIST (*National Institute of Standards and Technology*) MS database. The profile composition of the metabolites of the three cultivars 'Citra', 'Delima' and 'Madu Deli Hijau' from Boyolali Village, Demak Regency were then compared. Data dobtained was analyzed using UPGMA Clustering Method and SSM (*Simple Matching Coefficient*) in software MVSP 3.2. Determination of possible marker compounds for wax apple based on loading plots and PCA score plots using The Unscrambler X 10.4 and supported by descriptive data of compounds that have been analyzed for their presence.

RESULTS AND DISCUSSION

Morphology of Three Wax Apples Cultivars

The results of observations in Boyolali Village, Demak Regency showed that three cultivars of wax apples were found, including 'Citra', 'Delima', and 'Madu Deli Hijau' cultivars. The characterization results showed the existence of morphological diversity of the three wax apple cultivars. This research was conducted at the same location, so it has characteristics homogeneous environment. The diversity obtained is influenced by the existence of cultivar differences. Differences in genetic arrangement cause variations to be expressed in the phenotypes of each cultivar, so that when grown in the same environment, these cultivars will produce different phenotypic appearances (Sinay *et al.,* 2016).

Table 1. Morphological characteristics of three wax apples cultivars in this study

Characters	*S. samarangense* 'Citra'	*S. samarangense* 'Delima'	*S. samarangense* 'Madu Deli Hijau'
Stem circumference (cm)	48–65	60–74	38–59
Stem texture	Off the crust	Off the crust	Off the crust
Leaf shape	Ovate	Ovate	Oblong
Leaf length (cm)	20,2–22,8	20,53–22,2	21,2–22,43
Leaf width (cm)	8,7–9,97	8,17–9,3	6,9–7,8
Leaf area (cm^2)	124,92–156,5	121,79–155	99,67–121,08
Fruit shape	Bell shaped slighty rounded	Bell shaped	Bell shaped slighty rounded
Exocarp surface	Smooth and wavy	Smooth and flat	Smooth and wavy
Ripe fruit color	Dark red	Bright red	Green with a pink tinge
Fruit base diameter (cm)	3,24–3,44	2,36–2,67	3,36–3,45
Fruit tip diameter (cm)	5,35–5,69	3,95–4,08	5,43–5,76
Fruit height (cm)	6,06–6,49	5,02–5,15	6,59–6,76
Mesocarp thickness (mm)	15,3–16,63	7,67–8,4	10,05–11,3
Fruit weight (gr)	72,29–101,28	33,62–46,92	91,83–101,15
Taste of ripe fruit	Sweet	Slightly sweet	Slightly sweet
Texture of ripe fruit	2,57 (Crunchy)	2,24 (A bit crunchy)	3,09 (Crunchy)
The number of seeds	0–2	1–2	2–3
Seed width (mm)	15,93	10,1–16,9	14,66–17,26
Seed height (mm)	14,4	9,15–13,57	16,5–17,83

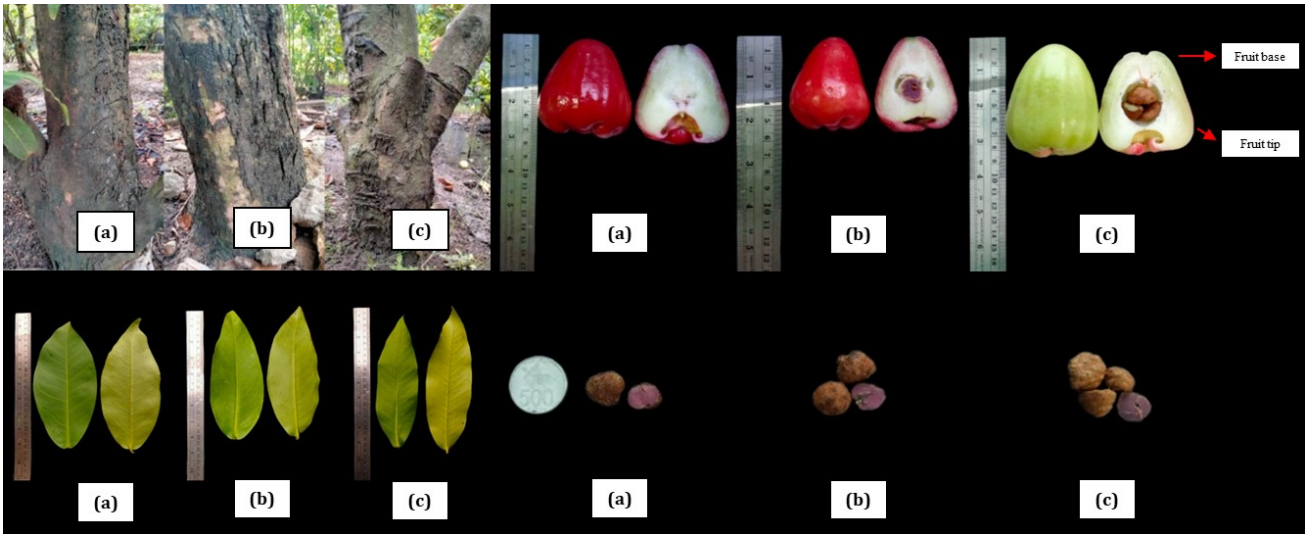

Figure 1. Stems, leaves, fruit and seeds of wax apples (a) 'Citra', (b) 'Delima', (c) 'Madu Deli Hijau' in this study

The stem morphology of the 'Delima' wax apple has the greatest circumference due to the age difference of 10 years, while the 'Citra' and 'Madu Deli Hijau' wax apples are 4 years old. Cambium activity causes secondary growth in stem diameter as the plant ages. Uthbah *et al*. (2017) stated that the age of the plant greatly affects the size of the stem diameter, the older the plant, the greater the diameter of the stem. Leaves of three wax apples have different sizes and shapes. The longer and wider the size, the greater the area. The leaves shape of the 'Citra' and 'Delima' wax apples are oval, while the 'Madu Deli Hijau' oblong. Shape variation can be used as a distinguishing morphological character. Widodo (2015) describes wax apple leaves as having short and thick stems with protruding, elongated to lanceolate leaves.

Wax apple fruit morphology has uniqueness especially in exocarp part. Exocarp of 'Citra' and 'Delima' have different color density levels. Exocarp of 'Citra' wax apple tends to be dark red, 'Delima' bright red and 'Madu Deli Hijau' green with pink bursts. Fruit color are the genetic characteristics of each cultivar, species and variety. In fruit development, the ripening of fruits results in color change owing to degradation chorophyls and varied pigment biosynthesis like anthocyanins in flesh or fruit skin (Kapoor *et al.,* 2020). 'Citra' and 'Madu Deli Hijau' wax apples have a bell shaped slighty rounded with a smooth and wavy exocarp, while 'Delima' wax apple has a bell-shaped fruit and an exocarp that tends to be smooth and flat. Widodo (2015) states that fruits of wax apples have varied colors and shapes. There is a difference in the weight of the wax apple fruit. The size of the 'Citra' and 'Madu Deli Hijau' are relatively large. This size has a positive correlation with fruit weight. Fruits with a large size will have a heavier fruit weight. Mesocarp is the part of the fruit that is consumed. 'Citra' wax apple has the greatest mesocarp thickness. Fruits with a thick mesocarp tend to have a higher percentage of edible parts (Febjislami *et al.,* 2018).

In addition to the thickness of the mesocarp, taste and texture components are things that consumers pay attention to when making a choice. Based on organoleptic tests, the panelists preferred 'Citra' wax apple because of its sweet taste and crunchy texture. 'Citra' wax apple has a sweet taste, thick, stiff flesh and is of great economic value (Widodo, 2015). Differences in the sweet taste of the three wax apple cultivars can also be influenced by panelist perceptions and the sugar content contained in the fruit. In addition, seasonal factors can affect the taste of fruit. The research was conducted during the wet season. The wet or rainy season can decrease the fruit's sweetness due to the high concentration water content in the environment, which increases the fruit's water content. Seeds are the reproductive organs of a plant. This research shows that not all 'Citra' wax apple accessions have seeds. This is likely due to genetic factors. A plant is considered to produce seedless fruit if it can produce fruit without absolute seeds, aborted seeds or a reduced number of seeds (Rahayu *et al.,* 2012).

Metabolite Profile of Three Wax Apples Cultivars

The results of the metabolite profile analysis showed that the three samples contained primary and secondary metabolites with different types, levels and amounts of compounds detected. This difference is because each plant can synthesize various kinds of organic compounds influenced by genetic and environmental factors. Variations in

the genetic composition of plants affect the emergence of cultivars which affect the process of metabolite biosynthesis so that various compounds are produced both qualitatively and quantitatively. Bakar *et al.*, (2016) stated that genetic factors (genus, species and cultivar) and environment (planting season and location) could affect phenolic levels and profiles.

Table 2. Metabolite profile of three wax apples cultivars in this study

S. samarangense **'Citra'**		***S. samarangense*** **'Delima'**		***S. samarangense*** **'Madu Deli Hijau'**	
Name of Compound	***Area (%)***	***Name of Compound***	***Area (%)***	***Name of Compound***	***Area (%)***
Butanoic acid, methyl ester	0,27	*Trimethylsilylmethanol*	2,66	*n-Propyl acetate*	3,26
2-Butanone, 4-hydroxy-3-methyl-	2,88	*2-Propenoic acid, 2-propenyl ester*	1,97	*Oxalic acid, cyclobutyl hexyl ester*	2,40
2,2'-Bioxirane	3,17	*2,2-Dimethoxybutane*	20,43	*2,2-Dimethoxybutane*	21,99
1-Propene, 3-methoxy-2-methyl-	3,49	*Desulphosinigrin*	0,43	*o-Acetyl-L-serine*	0,45
2,2-Dimethoxybutane	19,85	*Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester*	0,91	*Glyceraldehyde*	18,34
o-Acetyl-L-serine	0,46	*Glyceraldehyde*	16,06	*d-Ribo-hexos-3-ulose*	2,71
Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	0,86	*d-Ribo-hexos-3-ulose*	2,43	*1,3,3-Trimethoxybutane*	5,03
Glyceraldehyde	21,65	*1,3,3-Trimethoxybutane*	4,94	*2,3,4,4-Tetramethyl-pentane-1,3-diol*	4,53
DL-Arabinose	0,64	*1,3-Dioxolane-4-methanol, 2-ethyl-*	4,33	*5-Mercaptotetrazole*	1,54
2,3,4,4-Tetramethyl-pentane-1,3-diol	5,19	*Dihydroxyacetone*	19,91	*Dihydroxyacetone*	23,19
5-Mercaptotetrazole	1,57	*Nonane*	2,06	*Nonane*	2,03
Dihydroxyacetone	23,41	*d-Glycero-d-ido-heptose*	0,74	*1,2:5,6-Dianhydrogalactitol*	0,74
Nonane	2,49	*DL-Arabinose*	0,70	*DL-Arabinose*	0,86
D-Mannoheptulose	0,47	*Decane*	2,75	*Decane*	2,77
Decane	3,49	*L-Mannose*	10,67	*a-Pyrrolidinopentiophenone*	0,42
4H -Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0,88	*4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-*	1,03	*4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-*	0,74
Cyclotetrasiloxane, octamethyl-	0,18	*L-Glucose*	1,68	*d-Mannose*	0,98
a-d-Glucofuranosyl benzenesulfonate	0,12				
L-Sorbose	1,96				
Tetrasiloxane, 1,1,3,3,5,5,7,7 -octamethyl-	0,21				
1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	0,12				
7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	0,17				
9-Octadecenamide, (Z)-	0,35				

The metabolite profiles of wax apples 'Citra', 'Delima', and 'Madu Deli Hijau' revealed seven compounds detected at different levels in the three cultivars. These compounds include 2,2 Dimethoxybutane, a volatile compound that gives aroma to fruit. This compound also acts as an antimicrobial. Glyceraldehyde is a monosaccharide that plays an important role in carbohydrate metabolism and gives the fruit a sweet taste. DL-Arabinose is a reducing sugar which is classified as aldopentose. Dihydroxyacetone or commonly called glycerone is a simple sugar ketose (Baynes & Dominiczak, 2014). Nonane is an alkane group of hydrocarbon compounds. In berries, the distribution of alkanes plays a role in the appearance of fruit shine and plays a role in plant transpiration to control water loss (Klavins, 2020). Decane, a compound belonging to the alkane class, gives aroma and taste to fruit and has high antioxidant activity. 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl is a secondary metabolite of the ketone group and contributes as an aroma compound in fruit.

Relationship of Three Wax Apples Cultivars

Analysis of relationships was carried out to determine the relationship of a distant or close plant based on the same character (Azizah *et al*., 2019). The relationship among the nine accessions of wax apples based on morphological characters shows that the accessions of each cultivar can be grouped according to the type of cultivar, so that these accessions are located in the same group with different similarity indices. This shows that the accessions of each cultivar have many similarities because they come from the same lineage. The closer the similarity index to 1 have closer relationship (Kundariati *et al.,* 2021). Relatively small differences influence the difference in the high similarity index between accessions in quantitative characters. Quantitative characters are influenced by many genes that contribute little to the phenotype (Rosmaina *et al.,* 2021).

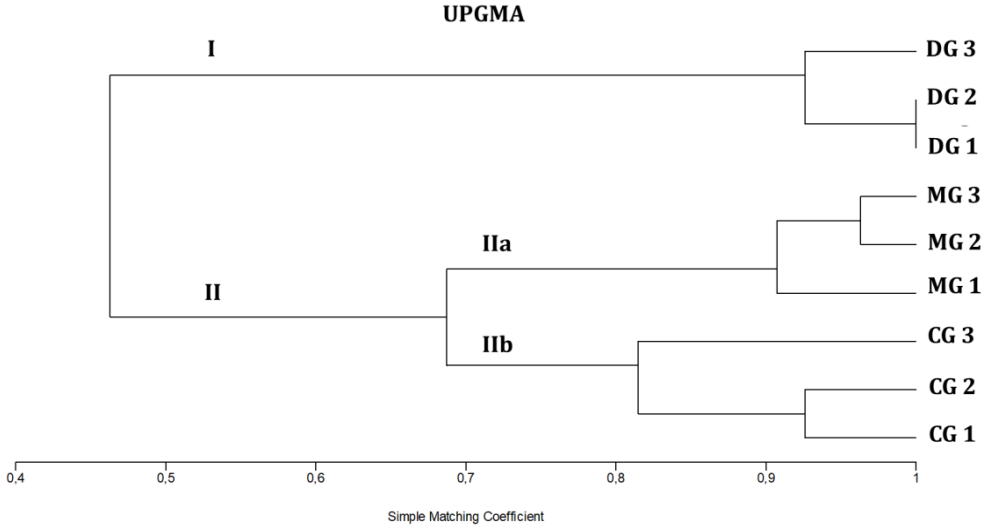

Figure 2. Phenogram of phenetic relationship based on morphological characters of three wax apples cultivars in this study (CG : 'Citra', DG : 'Delima', MG : 'Madu Deli Hijau')

The relationship of the three cultivars based on morphological characters can be seen in Figure 2**.** Phenogram analysis shows that the 'Delima' wax apple has the farthest relationship because it is separated from other cultivars by a similarity index of 46%. Relationships can be far if the similarity coefficient is less than 60% (Due *et al.,* 2019). The distinguishing characteristics of the 'Delima' wax apple that separate it from two other cultivars are stem circumference, fruit surface, exocarp color, fruit shape, fruit size (fruit tip diameter, fruit base diameter and fruit height), fruit weight, mesocarp thickness, fruit taste, fruit texture, number of seeds and seed size. Based on morphological characters, 'Citra' and 'Madu Deli Hijau' wax apples are grouped and closely related with a similarity index of 69% because they have more similarities including stem circumference, exocarp surface, shape, size of fruit (diameter of tip, diameter of base, height, weight), texture and seed size. The more similarities the morphological characters have, the greater the similarity index and the closer relationship.

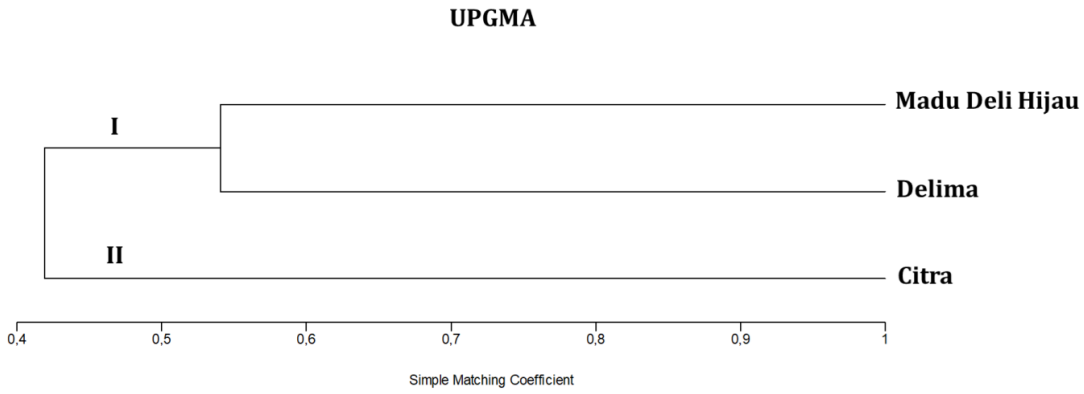

Figure 3. Phenogram of phenetic relationship based on metabolite profiles of three wax apples cultivars in this study

Based on the phenogram in Figure 3., it can be seen that 'Madu Deli Hijau' and 'Delima' wax apples clustered at a similarity index of 54% and were followed by the 'Citra' wax apple which was separated from other cultivars at a similarity index of 42%. The analysis shows that based on the metabolite profile, 'Madu Deli Hijau' and 'Delima' wax apples have the closest relationship. Both have similarities in several types of compounds and the number of compounds detected is almost the same. 'Citra' wax apple has the furthest relationship with other cultivars. This is because 'Citra' wax apple has the most compounds detected compared to other cultivars, so it has more differences in the types of compounds. The more differences characters you have, the more distant the relationship (Zulkifli *et al.,* 2023).

Phenogram of consanguinity based on morphological characters and metabolite profiles shows different grouping patterns. The differences in the grouping patterns may be due to the fact that not all metabolite profiles can interpret morphological characters. Research by Hanifah *et.al,* (2018) states that the metabolites detected in several eggplant accessions are not included in the regulatory process that leads to fruit phenotypes. Metabolites themselves are the result of the interaction of the system's genome with its environment and are not just the end product of gene expression, but part of the regulation of biological systems (Putri & Fukusaki, 2016). Another factor that can influence the grouping differences is the range of the number of morphological characters with the number of compounds detected in the three cultivars is not the same. Therefore, a combination of morphological characters and metabolite profiles was carried out as a determinant of the kinship of the three wax apples cultivars. The more characters used, the greater the likelihood of obtaining more accurate results.

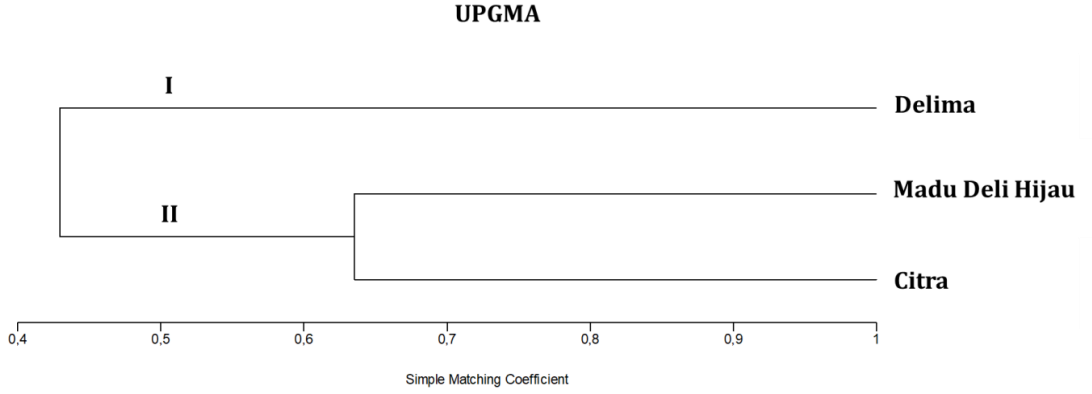

Figure 4. Phenogram of phenetic relationship based on morphological characters and metabolite profiles of three wax apples cultivars in this study

Based on the phenogram of combining the characters obtained (Figure 4.) it can be seen that 'Delima' wax apple has the furthest relationship with a similarity index of 43%, while 'Citra' and 'Madu Deli Hijau' wax apples have the closest relationship with a similarity index of 64%. This study showed that analysis of phenetic relationships based on metabolite profiles alone was considered to be less powerful in separating the three cultivars of wax apples. Therefore, it needs to be supported by analysis based on morphological characters. In order to produce a more accurate relationship, additional relationship analysis either DNA molecular markers or DNA Barcoding that are less influenced by external factors is required.

Autentication of Three Wax Apples Cultivars

The authentication of 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples from Boyolali Village, Demak Regency was carried out based on the observed qualitative characters. This is because the quantitative characters have a relatively small range so that they are less significant to be used as a benchmark in authentication activities. Meanwhile, the qualitative character of a plant is controlled by main or a few genes, which are relatively stable because its not influenced by the environment and can have a correlaton with character result (Ritonga, 2022).

Based on visual observations, the distinguishing character that can be used in the authentication of the 'Citra' wax apple is a dark red exocarp, in the 'Delima' wax apple is a bright red exocarp, the surface is smooth and flat also has a bell shaped fruit, while the 'Madu Deli Hijau' wax apple has an oblong leaf shape and green exocarp with a pink tinge. The morphological characters of the leaf and fruit organs of wax apples are unique, so these characters can be used as the main parameters in the authentication activities of the 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples based on morphological characters.

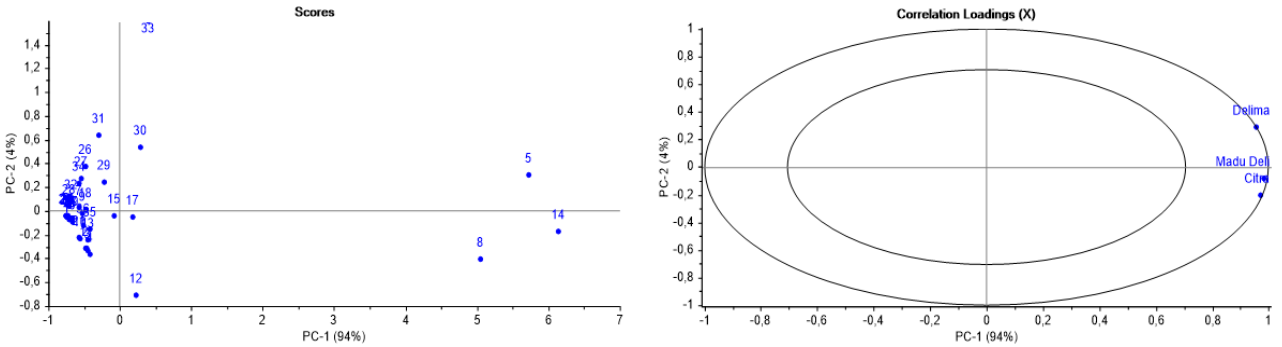

Figure 5. PCA analysis (a) score plot dan (b) loading plot 'Citra', 'Delima', and 'Madu Deli Hijau' wax apples extract in this study.

Authentication based on metabolite profiles was performed by comparing score plots and loading PCA plots (Figure 5.). The results showed that the marker compounds analyzed were 2,2 Dimethoxybutane in the 'Delima', Glyceraldehyde in the 'Citra', and Dihydroxyacetone in the 'Madu Deli Hijau'. These three types of compounds are the main compounds, namely the dominant compounds in plants that have a higher area compared to other compounds (Hakim *et al.,* 2018). These three compounds were detected in three cultivars with relatively small area differences (did not show much difference) so they were not significant when used in authentication activities. Therefore, a descriptive analysis is needed (Table 2) to determine the possibility of a marker compound in the form of an identity compound. The identity compound is a distinctive and unique compound found in a plant, which can be used as lead compounds (Aryantini *et al.,* 2020). Descriptive analysis showed that the marker compounds for 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples totaled 10, 7 and 5 compounds respectively.

Table. 2 Descriptive analysis of possible marker compounds of three wax apples cultivars in this study

S. samarangense **'Citra'**	***S. samarangense*** **'Delima'**	***S. samarangense*** **'Madu Deli Hijau'**
Butanoic acid, methyl ester	*Trimethylsilylmethanol*	*n-Propyl acetate*
2-Butanone, 4-hydroxy-3-methyl -	*2-Propenoic acid, 2-propenyl ester*	*Oxalic acid, cyclobutyl hexyl ester*
2,2'-Bioxirane	*Desulphosinigrin*	*1,2:5,6-Dianhydrogalactitol*
1-Propene, 3-methoxy-2-methyl-	*d-Glycero-d-ido-heptose*	*a-Pyrrolidinopentiophenone - d-Mannose*
D-Mannoheptulose	*L-Mannose*	
Cyclotetrasiloxane, octamethyl-	*L-Glucose*	
a-d-Glucofuranosyl benzenesulfonate	*1,3-Dioxolane-4-methanol, 2-ethyl -*	
L-Sorbose		
Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-		
7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane		

Based on the percentage of the largest area in the descriptive analysis of the compounds, the possibility of compounds that have prospects as marker compounds for the three wax apples fruit cultivars ranges from 3.17 to 10.67%. 'Citra' wax apple marker compound includes 2,2'-Bioxirane (3.17%) which is a compound resulting from the reaction between vegetable oils which have unsaturated bonds. This compound functions to prevent decay (Singh *et al.,* 2021) and has the potential as a antineoplastic (Shalini Ilango, 2021) and *1-Propene, 3-methoxy-2-methyl-* (3,49%) is a hydrocarbon compound that functions as a flavor enhancer in fruit. Marker compound of 'Delima' is 1,3-Dioxolane-4-methanol, 2-ethyl- (4.33%), which is a derivative of veleric acid which plays a role in maintaining the firmness of the plum fruit (Ponce *et al.*, 2010) and L-Mannose (10.67%) which is the primary metabolite of the monosaccharide class. In mangosteen fruit, this compound plays a role in fruit ripening (Mamat *et al.,* 2020). The marker compound of 'Madu Deli Hijau' is n-Propyl acetate (3.26%), a secondary metabolite compound of the ester group, which acts as an aroma compound and gives flavor to the fruit (Corradini *et al.,* 2016).

The results of the analysis of the metabolite profile of wax apples are still said to be possible marker compounds because there are still possible compounds that have not been detected in their entirety. This is because in this study the derivatization process was not carried out, which is a chemical process that aims to improve the volatility of a non-volatile compound so that it can be analyzed by gas chromatography (Hanwar *et al.,* 2015). Derivatization is able to increase detection ability of compounds that have been extracted before being put into an analysis machine such as GC -MS, so that all components of both compounds that play a role in the formation of aroma, taste and color in the wax apple fruit can be detected.

CONCLUSION

There was a diversity of morphological characters from the three cultivars wax apples. The profile of metabolites detected in the three wax apple cultivars has variations and total metabolites in the 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples amounted to 23, 17 and 18 compounds respectively. Based on the combination of morphological characters and metabolite profiles, the 'Madu Deli Hijau' and 'Citra' wax apple have the closest relationship with 64% similarity index. Morphological characters that can be used in authentication of 'Citra' wax apple (dark red exocarp), 'Delima' wax apple (bright red exocarp, smooth and flat surface and has a bell-shaped fruit shape),'Madu Deli Hijau' (elongated leaf shape and exocarp is green with pink streaks). The possible metabolite profiles that can be used in the authentication of 'Citra', 'Delima' and 'Madu Deli Hijau' wax apples total 10, 7 and 5 compounds respectively.

ACKNOWLEDGEMENT

We thanks to Mr. Rohmad for allowing to take samples of wax aplle from his garden and Eva Khoirun Nisa, M.Si for their helpful statistic discussion in preparing this paper.

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